

THE INSTITUTE OF
VETERINARY PATHOLOGY, UNIVERSITY OF ZUERICH
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**OBSERVATIONS ON THE
GENUS PROTEUS WITH
PARTICULAR
REFERENCE TO
INCIDENCE
PATHOGENICITY,
'TREATMENT
AND AGGLUTINATION
REACTIONS.**

THESIS
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INTRODUCTION

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INTRODUCTION

Organisms of the **Genus Proteus** are known to occur in the alimentary tract of man and some animals. Little is known however about the actual extent of their occurrence in the various species of animals.

The question of the pathogenicity of the genus in man and animals is in some doubt. This is particularly so of animals. There appears to be more evidence to support the theory that the organism is, under certain conditions, pathogenic for man.

My attention was directed to this organism by the natural occurrence of the infection in turkeys determined on routine examination of birds brought in to the State Diagnostic Laboratory for diagnosis. The history of the outbreaks and other relative information were obtained from the owners.

The pathogenicity of some of the strains isolated was tested by artificial inoculation, for feeding of experimental animals.

Attempts to evaluate the effect of antibiotics on the organism were made **in vivo** and **in vitro**. Few reports are available on suitable dosage or routes of inoculation for young birds. In vitro determinations with the antibiotics were made by visual examination of inhibition of cultural growth.

The incidence survey was made on animals from premises in the vicinity of Auburn, the same technique of sampling and cultivation being adopted in all cases. At least one hundred animals from each of the species were examined. They were from various premises. At the same time some tentative observations were made on the occurrence of other enteric organisms and the occurrence of certain cross agglutinations with this genus.

LITERATURE

Bergey (1) reports **P. retigeri** as occurring in cholera-like epidemics in chickens and gastro-enteritis in humans.

Biester (2) reports that *Proteus* infection occasionally occurs in young poults and may account for heavy losses, such infections being usually secondary to some environmental setback.

Mayfield (3) of the Mayfield Laboratories, Charles City, Iowa, has recently reported the isolation of **Proteus** from a number of outbreaks of disease in turkeys.

Packer (4) in a personal communication to Merchant reports the occurrence of **P. ammoniae** in animals, and Merchant, Packer, and Allen (5) report one clinical case of bovine mastitis due to **P. hydrophilus**.

Gwatkin (6) did not recover **Proteus** from cloacal swabs from apparently healthy chicks.

Harrison and Hansen (7) comment that little has been reported on the bacterial flora of the cecal feces of healthy turkeys. Feces from twelve turkeys were examined, all five months or more of age. Members of the genus **Proteus** were not recovered on any of the several media used. Anaerobic lactobacilli were the most numerous bacteria found.

Shapiro and Sarles (8) discussing the microorganisms in the intestinal tract of normal chickens say that when not fed for twenty-one hours, newly hatched chicks harbor few organism in the tract. **Lactobacilli** appear to be the most numerous group except in the colon. **Str. fecalis** are in large numbers in the ceca and colon. **E. coli** is the predominant coliform.

Craigie (9) reported the isolation of **Proteus** from 101 dogs and considered it to be the cause of peritonitis and omphalitis in the new-born, acute diarrhoea in puppies, and a sub-acute dysentery in dogs over three months old. Discussing this report, Gorman (10), said he considered **Proteus** a part of the normal intestinal flora of dogs. He did not claim that this invalidated Craigie's work. In a later paper Craigie (11) discusses Gorman's observation. He considers it probable that many of these so called normal dogs actually were subject to attacks of diarrhoea or showed obscure symptoms that might be referable to regional enteritis. In a series of 250 dogs selected at random he found an incidence of 40%. Before Gorman's 100% incidence can be accepted, the possibility of recent infection, as in a veterinary hospital must be elimi-

nated; in other words, the dog might have been placed in a contaminated kennel or picked up *Proteus* organisms as a temporary intestinal inhabitant from some other source in the hospital. It must be made clear that dogs were sampled from a wide segment of the dog population rather than from one small group which might show a higher incidence than normal. Craigie suggests that one hundred dogs could be taken from a certain kennel that will be unnamed and 100% infection with **Proteus**, **Giardia**, and **Coccidia** be found.

Organisms of the genus *Proteus* are considered by many to be nonpathogenic. It is apparent, however, that there are a number of reports suggesting that under certain conditions the genus plays an important part in the causation of disease in animals.

Dubos (12) says that **P. vulgaris** causes 6 to 13 % of the human urinary tract infections and in some cases it has been isolated from cases of gastro-enteritis where it played an etiologic role. **P. morganii** has been isolated from a number of outbreaks of infantile diarrhoea and from the stools of normal persons.

Jordan (13) mentions a variety of conditions in man in which the pathogenicity of the organism can hardly be doubted. As a producer of cystitis it probably ranks next to **B. coli**. Further evidence is needed to establish the role of **Proteus** in food poisoning. Animal inoculation shows that a great range of virulence exists among the **Proteus** cultures that have been tested. Freshly isolated strains from pathological sources may produce definite lesions including abscesses, enlargement of the spleen and a diarrhoeic condition.

The Medical Research Council (14) indicates that the organism occurs frequently in association with other bacteria in inflammatory conditions in man, and that it rarely occurs as a primary agent, although reporting that Metchinkoff and his associates believed a *Proteus* to be a primary cause of infantile diarrhoea.

Fulton and Harrison (15) suggest that the presence of **P. ammonia** in the urinary tract of man associated with clinical conditions is worthy of careful study. They do not agree that this organism is synonymous with **P. mirabilis**. The same authors (16) indicate that it is becoming apparent that *Proteus* species have more importance in relation to various disease processes than they have traditionally been assigned.

Cherry, Lentz, and Barnes (17) isolated strains of **P. mirabilis** that were apparently identical biochemically and

serologically from ham, and from nine of nineteen patients who became violently ill with gastro-enteritis after eating the food. Attempts to demonstrate a causal relationship of staphylococci or streptococci were unsuccessful. It is believed that the studies furnish strong circumstantial evidence of the probable etiological relationship of **P. mirabilis**. Although the disease was severe it was of short duration. The average length of illness of eighteen patients was forty hours.

Cope and Kasper (18) in their serilogic study of **P. rettgeri** demonstrated antigenic factors in III strains of *P. rettgeri* and similar Gram negative bacilli isolated from human fecal specimens.

In discussing the pathogenesis of canine dysentery Craigie (19) says that puppies infected at birth or shortly afterward may die within a few days of omphalitis and peritonitis in which **Proteus** is the only organism that can be recovered. He has not seen this syndrome with any other intestinal infection. Chewing fits in which the puppy salivates, works his jaws, stretches out in spasms, and finally goes into a running fit are frequently encountered with **Proteus**, **Spirochaetea**, **Giardia**, and coccidia. Although mature dogs may develop acute dysentery and chronic dysentery, another syndrome is frequently observed. This he named chronic regional enteritis, since on autopsy only a small portion of the small intestine was found to be inflamed. This is typically caused by **Proteus**, but other organisms may be involved as secondary infections. Although **Proteus** organisms have been found in apparently healthy dogs, this infection has usually been fleeting and the organisms could not be isolated on subsequent examinations. Craigie has never observed a persistent *Proteus* infection in which there were not some symptoms of disease.

It is interesting to note here an observation by Jordan and Burrows (20) in which they draw a somewhat similar conclusion in regard to the *Pneumococcus* in man. They say that the percentage of carriers is frequently high, but the carrier state is not permanent but rather sporadic and intermittent. Discussing the treatment of dogs, Craigie (21) says that although streptomycin is specific for acute **Proteus** infection it is not entirely satisfactory in these cases since the symptoms recur as soon as the streptomycin is eliminated from the body, and after several courses of treatment, the organism becomes resistant to the streptomycin. In his hands, the most satisfactory treatment has been the use of autogenous bacterins. Autogenous bacterins have been prepared

and used on about 150 cases of chronic **Proteus** Dysentery about 95% of these have shown more or less improvement. Many of them were chronic cases that had made the rounds of several veterinarians before the bacterin was used and had regained normal health and remained normal afterward, some as long as two years. The few which died during the course of treatment, it is felt, would have died anyway. In a few cases the bacterin failed to eliminate the organism from the stool although it did cause some improvement in the general condition. There were no serious reactions to the bacterin therapy. Although no antigenicity studies have been made with these organisms, it is probable that they have a complex antigenic structure similar to the Salmonella group. Heterogenous bacterins were tried without success on several cases where the owner could not afford to have an autogenous bacterin prepared. Recently, six known effective bacterins were combined and used in these cases. The results seem to be better with this mixed bacterin. It is earnestly hoped that some biological company will investigate this problem and will produce a mixed **Proteus** bacterin which will be effective in a large percentage of cases, eliminating the hazard of autogenous bacterins.

Dubos (22) reports that in man sulphonamides have only a limited value in treatment of infections caused by **P. vulgaris**. Streptomycin has proved useful in many cases.

Ordal and Meyer (23) have carried out experiments on the effect of streptomycin on **Proteus** infections of the chick embryo. They conclude that **P. vulgaris** produces a rapidly fatal infection in eleven day old chick embryos whereas those sixteen days old are relatively resistant. Streptomycin in doses of one thousand units or more is necessary to arrest eighteen hour infections. It is more effective when administered six hours after infection. Larger doses administered early not only gave protection but in addition eliminated the organism from the embryo.

In regard to the dosage of streptomycin for chicks, Benos (24) found that it was relatively non-toxic to chicks, 964 units per gram of live weight having been given within a fourteen-hour period without any marked ill effects. He found it effective against **S. pullorum**. The dosage was 2,500 to 5,000 units subcutaneously commencing sixteen hours after infection. In an evaluation of aureomycin for intestinal antiseptics, Metzger and Shapse (25) say that coliform organisms were reduced, but there was rapid proliferation of **Proteus** organisms. Whether or not **Proteus** organism alone or in mixed

flora are dangerous in large bowel surgery from a pathogenic viewpoint is still to be determined.

For the identification of the original strains, I was guided by the classification of Bergey (26) and the cultural work of Rustigian and Stuart (27) and Christensen (28). Using the methods described the addition of immune serum to the medium for the production of individual colonies as advocated by Sompolinsky (29) seems to be unnecessary.

Examination of the literature revealed a number of interesting reports on the occurrence of cross agglutination with the genus *Proteus*. For example, Gwatkin (30) isolated a strain of *Proteus* from a hen which reacted to variant but not regular pullorum antigen and produced an antiserum in a horse which agglutinated the yonine variant but not the regular form of *S. pullorum*. The use of this serum provided a clear-cut method of differentiating variant from regular forms of *S. pullorum*. This *Proteus* stimulated agglutinins against variant pullorum antigen in rabbits, chickens, horses and cows but not in guinea pigs.

The antigenic relationship between *Proteus* and *Brucella* in swine has been studied by Kamel (31). He reports that *Proteus* ox 19 seems to share an antigenic factor with *Br. suis*. It is agglutinated by *Brucella* immune serum and can absorb its agglutinins from it as well. *Br. suis* is agglutinated by *Proteus* ox 19 immune serum and can absorb its agglutinin from it. Of 198 swine sera 178 agglutinated *Proteus* ox 19. These antibodies are due to infection of swine with this organism or to latent infection with *Brucella*. The prevalence of swine brucellosis in the area indicated a probability that antibodies against ox 19 were due to the serological relationship between it and *Br. suis*.

Calder (32) examined a large group of human patients presumably suffering from chronic brucellosis. Agglutinins for *P. tularensis* were seldom encountered, but for *Proteus* they were frequently noted. There is apparently an antigenic relationship between the two. A reaction should arouse suspicion of brucellosis. In practice if repeated tests are made, *Proteus* agglutinins do not rise in brucellosis with passage of time. The antigenic similarity of the two organisms seems to rest in the somatic rather than in the flagellar component of the *Proteus* cell.

Foshay (33) says that antibrucella serums may have very broad agglutinating properties for organisms of other genera and families, including most of the colon-typhoid dysentery

group, some *Salmonella*, some *Aerogenes*, and even *Proteus*, and of *Alkaligenes*, *B. tularensis* and even *H. influenzae*.

When studying the incidence of *Proteus*, advantage was taken of the opportunity to observe the occurrence of organisms of the *Salmonella* genus.

Galton (34) in an examination of 3,600 anal swabs from dogs in Florida found an overall average of 30% to be carriers of *Salmonella*.

Gruikshank and Smith (35) gave the following results of examinations of small animals in London.

500 Dogs	<i>Salmonella</i>	5	0.8 %
	<i>Proteus</i>	139	27.2 %
	<i>Paracolon</i>	54	10.8 %
	<i>Ps. pyocyaneus</i>	6	3.2 %
500 Cats	<i>Salmonella</i>	7	1.4 %
	<i>Proteus</i>	105	21 %
	<i>Paracolon</i>	289	57.8 %
	<i>Ps. pyocyaneus</i>	0	0 %
133 Pigeons	<i>Salmonella</i>	3	2.25 %
	<i>Proteus</i>	11	7.3 %
	<i>Paracolon</i>	18	13.0 %
	<i>Ps. pyocyaneus</i>	0	0 %

Wolf, Henderson, and McCallum (36) found *Salmonella* in eighteen of one hundred rectal swabs from dogs at Michigan State Veterinary Clinic.

Of feces from seventy-one dogs in the Ohio State University Veterinary Clinic, Kinner (37) found thirteen, or 18 positive for *Salmonella*. Almost 50% of the dogs suffering from distemper were infected.

ISOLATION AND IDENTIFICATION.

The routine method of primary isolation adopted is to inoculate portions of tissues such as liver, heart, or intestine into selenite F broth. After eighteen hours incubation at 37° C. a loopful of this broth is streaked on desoxycholate plates. These are incubated for twenty-four hours. *Proteus* sp. grow well on this medium, frequently in isolated colonies which are considerably larger than *S. pullorum*, *S. gallinarum*, and other *Salmonellae*. Rarely quite small colonies are seen which might be compared with *Pullorum*. The colonies develop quicker than *Pullorum* and are smooth with an amber tint. There is little tendency for the organisms to spread on this medium. This seems to be a more simple method of obtaining isolated colonies than that necessitating the use of immune serum.

Typical colonies off the D.C.L.S. plates are inoculated into nutrient broth which is incubated for twenty-four hours. Sugars and other test media are inoculated from this. The sugars are composed of a nutrient broth containing 0.3% beef extract and 1% carbohydrate.

Urease production which is characteristic of this group was always noted in urea broth. However, other Gram-negative intestinal bacteria which are capable of splitting urea cannot do so in this medium because of the small amount of nutritive material and increased amount of buffer present. Christensen (28) devised the Urea Agar Medium in which he included peptone and dextrose and reduced the buffer content. Organisms of the paracolon group will grow on this medium. *Proteus* attacks the urea rapidly and after twenty-four hours incubation the color change due to ammonia production has penetrated deep into the medium. After twenty-four hours incubation the entire butt is alkaline in reaction. Urease-positive paracolons, in contrast, hydrolyze urea much more slowly showing only slight penetration of the alkaline reaction into the butt of the medium in six hours, and requiring three to five days to change the reaction of the entire butt. This is a useful medium to use when paracolon types are suspected.

A compound medium known as SIM is also useful to determine the presence of sulphides, indol, and motility.

Borrey's method of classification of this group was used;

It is as follows :

1. Mannitol negative

<i>P. vulgaris</i>	Sucrose	A G
	Maltose	A G
	Indole	—
<i>P. mirabilis</i>	Sucrose	A G Delayed
	Maltose	—
	Indole	—
<i>P. morgani</i>	Sucrose	— (Ordinarily)
	Maltose	—
	Indole	—

2. Mannitol positive (acid)

<i>P. rettgeri</i>	Sucrose	A Delayed
	Maltose	—
	Indole	—

The organism isolated here in 1949 from turkeys and chickens was regularly mannitol and maltose negative and fermented sucrose only after a number of days incubation. Glucose, xylose, and trehalose were regularly fermented with the production of acid and a small amount of gas. Arabinose, inositol lactose, dulcitol and rhamnose were negative. Five strains were indol negative. It would appear that most of our strains conform to Bergey's description of ***P. mirabilis***.

GROWTH OF PROTEUS ON SOLID MEDIA

This experiment was designed to determine the difference in the amount and type of growth of **Proteus** obtained on some of the various special types of agar used in the determination of enteric organisms.

A loop full of twenty-four hour broth culture of **Proteus** was streaked on each petri plate tested and the latter were incubated for twenty-four hours.

1. Bismuth Sulphite Agar—has been recommended for isolation of **Salmonella typhosa** and other members of the *Salmonella* group. The media contains dextrose.

Typhoid organisms from black colonies while gram positive bacteria and members of the coliform group are inhibited.

Proteus growth. Many discrete colonies 1/8 inch in diameter, black in color and surrounded by a clear ring.

2. Tryptose Agar—approximately the same amount of growth of **Proteus**. Colonies were a little smaller than on Bismuth sulphite. They were white, glistening with a cream colored center.

3. E.M.B.—containing Peptone, photone, phosphate, and two sugars, lactose and saccharose. Eosin and methylene blue are the indicator dyes.

The **Proteus** colonies were much smaller than on the above mentioned media. They were greyish-white in color and were surrounded by a cloudy zone.

4. Desoxycholate-citrate-agar — coliform organisms produce deep pink opaque colonies. Organisms of the *Salmonella* group produce colorless colonies.

Proteus grew only on the first line of the inoculation. Discrete glossy translucent colonies were produced.

5. Desoxycholate lactose sucrose agar—this is a non-selective primary plating medium for the isolation of enteric pathogens. Organisms other than those of the enteric group are inhibited.

Of the five media tested, the heaviest growth of **Proteus** was on this media. There was however no difficulty in obtaining isolated colonies. These were grey in color, the larger colonies having a brownish center.

OCCURRENCE IN THE FIELD IN POULTS IN 1949

The first outbreak of disease from which the organism was isolated occurred at the end of April, 1949. The hatch consisted of 2,500 poults. At the time of the investigation the birds were fifteen days old, 110 had died and there were about 400 sick. On another farm 500 poults from the same hatchery were involved. Deaths were reported at the rate of five a day from the date of arrival. Two other outbreaks were recorded in April, both in poults from a few days to two or three weeks of age. In one case **Pullorum** was also isolated.

One outbreak was recorded in May. 1,250 poults were involved, the age of two groups examined being two weeks and four weeks respectively. In this outbreak a predisposing factor was considered to be the presence of litter composed of shavings in the gizzards of many of the birds. The mortality was about 10%.

Five outbreaks were recorded in June. In the first outbreak the age of the birds at the time of the investigation was four weeks. **Pullorum** was also isolated in this outbreak and there was some evidence of litter eating. The mortality was over 10%. In one of the other four outbreaks there was considerable evidence of shavings in the gizzards of a number of birds and a heavy mortality was reported.

Two outbreaks were recorded in July. In one the turkeys were ten weeks old. We studied this latter outbreak for some time but were unable to find any other contributing factor.

OCCURRENCE IN CHICKENS IN 1949

When dealing with suspected outbreaks of Salmonellosis or enteric infections in chickens and turkeys the same bacteriological procedures are adopted.

In July the **Proteus** organism was isolated from three out of five hens received for examination as suspected **Pullorum** reactors. The birds were all from the same farm.

Isolations were also made from two groups of birds from another farm. The birds in one group were ten days old and in the other group thirteen weeks old.

Another isolation of the organism was made from young chicks and from this outbreak a variant of **Pullorum** was also isolated.

Two isolations were made in August, in one case from chicks and another from adult birds. In the latter case there was a history of previous moderate coccidiosis. In the former the deaths commenced when the chicks were five days old and in two weeks 150 died out of 1,000.

The following are cases gleaned from reports in the State Diagnostic Laboratory in 1950 in which **Proteus** sp. was apparently involved :

1. Owner—Young, Enterprize, Alabama, 13 July.
Species—Avian, chicks.
Mortality—50 to 75 per day. 700 out of 2100 to date.
Lesions—Necrotic areas and abscesses in lung and air sacs.
Age—14 days
Aspergillus fumigatus and **Proteus** isolated.
2. Owner—Hickman, 29 No., 1949
Species—Avian, hen
Check for **Pullorum**
Plate test and tube test positive
Proteus only isolated
3. Owner—Edwards, 5 June, 1950
Avian, pheasants
Losses—40 out of 400
Proteus only isolated
4. Kali Oka Farm, Florida
Avian, turkeys
5. Chinchilla Farm, Columbus, Georgia
1 Chinchilla found dead in pen
Proteus and **E. coli** only isolated

6. Jordan
Bovine—epidemic diarrhoea
Specimen of faeces. **Proteus** only
7. Bacteriological examination of eight suspected **Pullorum** reactors did not reveal **Pullorum**.
Proteus sp. was isolated from one case.
8. Owner—Blount
Species—Avian, chicks
5,000 in flock
Deaths—15 on first day, 15 on third day, 50 on
on fourth day.
Vaccinated intranasally against Newcastle on
first day. Symptoms and lesions suggested
Newcastle disease.
Proteus isolated in pure culture
9. Owner—Ivey
Species—Avian, chicks
Age—three weeks old
Vaccinated against Newcastle disease
Some symptoms of Newcastle disease
10. Owner—Pollard
Species—Avian, turkeys
Age—fifteen weeks
40 dead from 4,000. Toxicologist reported traces
of arsenic.
Proteus isolated in pure culture
11. Owner—Parker
Species—Avian, chicks
3,500 in flock
Intranasal vaccinations against Newcastle disease
at day old
Food contained sulpha drug
Mortality—8th day—100
 9th day—100
 10th day—100 Sulmet given
 11th day—300
 13th day— 40
The chicks examined showed no respiratory symptoms.
There were signs of a white diarrhoea.
On post-mortem there were no gross lesions.
Proteus sp. was isolated from each of the four chicks
which were killed for post-mortem examinations.

The isolation of the organism from young chicks is interesting in view of Gwatkin's (6) observation that **Proteus** sp. is not a normal inhabitant of the intestinal flora of chicks and also other reports on the normal intestinal flora of chicks and poults, indicating that **Proteus** sp. does not occur.

INCIDENCE IN VARIOUS SPECIES OF ANIMALS

There is little information available relative to the occurrence of **Proteus** sp. in the various species of animals, healthy or otherwise.

Apart from Craigie's work in dogs the organism has not been reported as pathogenic in these animals and its occurrence has therefore been ignored. As we have already seen, however, some idea of its incidence has been suggested by information revealed in reports made by workers on the Salmonellosis.

Similarly one can obtain little data on the incidence in chickens and turkeys by reference to the literature. Even less information is available concerning the occurrence of **Proteus** sp. in the intestinal tract or elsewhere in other species of animals.

The method of obtaining the fecal samples was the same throughout. No attempt was made to sterilize the external orifice. The specimen was obtained by passing a sterile swab into the rectum and the swab was then returned to the sterile container. Immediately on reaching the laboratory the swabs were washed off into tubes of Selinite broth. These were incubated for twelve to eighteen hours at 37°C. and the cultural procedure already described carried out.

Dogs. Specimens were taken during the spring and summer months from one hundred dogs. All the dogs were seen at the Small Animal Clinic of the School of Veterinary Medicine, Auburn, Alabama.

The specimens were of a random nature in that dogs in various phases of health were examined. Some attempt was made, however, to obtain samples from dogs showing enteric disturbance. In some instances the dogs were apparently healthy, many of these being recovered surgical cases. Some of the dogs were pound dogs or boarders and complete histories are therefore not available for all cases. The results are shown in Tables 1 and 2, along with pertinent information from the case histories that were available.

Proteus sp. was isolated from forty-five dogs. Sixteen of these were from animals with diarrhoea or intestinal parasites. Of the fifty-five specimens which were negative for **Proteus**, eight were from dogs with diarrhea or intestinal parasites. The organism was isolated from animals of various ages.

The organisms which were not **Proteus**, and still non-lactose fermentors were examined at the Salmonella Typing Laboratory of the University of Kentucky. **Salmonella schottmulleri** (animal strain) was the only member of this genus to be identified. **Pseudomonas Aeruginosa** was identified in one case. The other slow lactose fermentors were of the para-colon group but the identification was not completed. One of these was biochemically but not antigenically similar to the Arizona paracolon group. Organisms of this latter group have recently been reported as causing considerable disease in chickens and poults in the Southeastern states.



Table 1
DOGS FROM WHICH PROTEUS WAS ISOLATED

Breed	Age	Sex	Condition	Treatment	Result
1.	Young				Proteus
2.	Young				Proteus
3. Cocker	10 wks.		Distemper, Hooks Rounds, Bloody faeces	Distemper serum Tenta- tive Antibact. serum	Proteus
4. Cocker	10 wks.		Same as No. 3		Proteus
5. Cocker	3 mos.		Tapeworm	Distemper immunization	Proteus
6. Boxer	9 wks.		Hooks & Round worms	None	Proteus
7. Beagle	6 mos.		Distemper, Hooks, & Round worms. Blood on swab.	Serum Penicillin	Proteus
8. Pound	Puppy ..				Proteus
9. Pound	Puppy ..				Proteus
10. Pound	Puppy ..				Proteus
11. Pound	Puppy ..				Proteus
12. Pound	Puppy ..				Proteus
13. Doberman	3 mos.	M	Hookworms	FesO4	Proteus
14. Cocker	2 yrs.	F	Diarrhoea	Strepto. Penic	Proteus
15. Cocker	4 days	M	Diarrhoea	Strepto. Penic.	Proteus
16. Cocker	4 days	M	Diarrhoea	Strepto. Penic.	Proteus
17. Cocker	4 days	M	Diarrhoea	Strepto. Penic.	Proteus
18. Hound	Adult	M	False joint	Surgery	Proteus
19. Cocker	1. yr.	M	Mange	Canex	Proteus

Breed	Age	Sex	Condition	Treatment	Result
20. Setter	3 yrs.	M	Pyometra	Streptomycin	Proteus
21. Cocker	3 wks.		Ankylostomes		Proteus
22. Cocker	3½ mos.	M	Distemper	Aureomycin, Peni.	Proteus
23. Cross	3 mos.	F	Mange	Canex	Proteus
24. W.H. Terrier	2 yrs.	M	Diarrhoea	Bacitracin	Proteus
25. Pointer	4 yrs.	F	Fracture	Penicillin	Proteus
26. Setter			Distemper	Sol. B	Proteus
27. Hound	5 mos.	M	Distemper	Penicillin	Proteus
28. Pup			Hard Pad		Proteus
29. Spaniel	3 yrs.		Diarrhoea	Bacitracin	Proteus
30. Pound					Proteus
31. Found					Proteus
32. Pound					Proteus
33. Pup	5 wks.				Proteus
34. Pup	3 wks.				Proteus
35. Hound	Adult		Pneumonic	Penicillin	Proteus
36. Hound	Adult		Amputation		Proteus
37. Hound	Adult		Fracture		Proteus
38. Cocker	Adult	F	In whelp		Proteus
39. Hound	4 mos.	M	Distemper	Penicillin Sol B.	Proteus
40. Hound	1 yr.	F	Spay		Proteus
41. Terrier	14 yrs.	F			Proteus
42. 717			Diarrhoea	Bacitracin	Proteus
43. Dachsund			Ankylostomes	Aureomycin	Proteus
44.			Chochiomiasis		Proteus
45.			Fracture		Proteus
46.			Wound		Proteus

Table 2
Dogs FROM WHICH PROTEUS WAS NOT ISOLATED

Breed	Age	Sex	Condition	Treatment	Result
1.	Young				Negative
2.	Young				Negative
3.	Young				Negative
4. Cocker	3 mos.	Nil		Rabies Vaccine Homo. serum	Lactose Fermentor
5. Cross	6 mos.	Fracture setting		None	Lactose Fermentor
6. Beagle	6 mos.	Distemper		Serum Penicillin	Lactose Fermentor
7. Beagle	6 mos.	Distemper		Serum Penicillin	Negative
8. Hound	4 mos.	F	Distemper, coccidia Hooks & Round worms		Negative
9. Hound	4 mos.	F	Distemper, Hooks & Round worms		Lactose Fermentor
10. Cross	5 mos.	M	Boarder, slight cough	Sulmeb Homo. serum	Lactose Fermentor
11. Collie		M	Malnutrition Coccidiosis	Sulmeb	Negative Not identified by Typing Lab.
12. Dachsund	20 mos.		Ear mite		Negative
13. Fox Terrier		F	Fleas & Hooks		Negative
14. Cocker	3 yrs.	M		Homo serum	Negative
15. 1712					Not identified
16. Pointer 1751	1 yr.	M	Pneumonia	Penicillin	Not identified

Breed	Age	Sex	Condition	Treatment	Result
17. Pointer 1750	1½ yrs.	F	Encephalitis	None	Negative
18. Collie 1810	10 wks.	M	Distemper	Penicillin Aureomycin	Paracolon
19. Hound 1673	7 mos.	M	Fracture	Penicillin	Negative
20. Setter	8½ mos.	M	Distemper	Aureomycin	Lactose
21. Pointer	6 mos.	M	Wound	Penicillin	Negative
Pound Pups					
22. 1					Negative
23. 2					Negative
24. 3					Negative
25. 4					Negative
26. 5					Negative
27. Hound	16 mos.	M	Mange		Negative
28. Collie	5 mos.	M	Distemper	Sulmet Penicillin	Negative
29. 1A					S. para.B
30. Boston	7 mos.		Distemper	Sulphathal.	Negative
31. Hound (1950)	5 mos.		Distemper	Penicillin	Identifi cation not completed
32. Foxhound			Bloody Diarrhoea	Parasites	Negative
33. Pound dog					Negative
34. Pound dog					Negative
35. Pound dog					Lactose
36. Greyhound pup					Lactose
37. (1)					Lactose
38. Collie pup					Negative
39. Hound			Distemper		Negative
40. Trixie	3 yrs.	F			Negative

Breed	Age	Sex	Condition	Treatment	Result
41.	3 mos.	F	Spay		Not Identified by Typeing Lab.
42. Pup	3 wks.				Lactose
43. (4) pup	3 wks.		Biochemically Ariz.		Paracolon but not typical antigenically
44. Shepherd	13½.hrs.	F	Leptospirosis	Streptomycin	Negative
45. 721			Parasites		Negative
46. Cocker			Taemasis		Negative
47. Pup (Sandford)					Negative
48. Pup					Negative
49. Cocker	4 days	M	Diarrhoea	Streptomycin	Negative
50. Cocker H.L. Johnson 3 Jan. & Feb.					Ps. Aeruginosa
51. Hound					Lactose
52. Boxer	3 mos.	M	Bloody Diarrhoea		Coliform
53. Dachshund	8 mos.	F	Bloody Diarrhoea		Coliform
54. Cocker 578		F	In Whelp		Not Identified
55.			Ulcers		Negative

Hogs. Rectal swabs have been examined from 100 apparently normal swine from eight different premises. The specimens were collected from swine of various ages. The incidence of *Proteus* sp. was eleven per cent. Six of these positive cases were from pigs about two months of age in a herd which was garbage fed. *Pseudomonas aeruginosa* was isolated in one case. No *Salmonella* were isolated.

Cattle. Specimens from apparently healthy cattle were taken from six different premises. Eighty four cows, three yearlings, and thirteen calves were included. Of these hundred specimens, *Proteus* was isolated from only three animals. Four plates revealed lactose fermentors. No *Salmonella* or paracolon types were isolated.

Chickens. Cloacal swabs from one hundred ten apparently healthy chickens were examined. As indicated in Table 3, *Proteus* was isolated from 14.55 % of the specimens. No *Salmonella* organisms were isolated.

Table 3
INCIDENCE IN CHICKENS

No. Examined	Age	<i>Proteus</i> Positive	Negative
6	Hens	0	6
17	Hens	3	14
32	Broilers	6	26
31	Pullets	7	24
24	Chicks (3 day old)	0	24

It will be seen that the incidence is much higher in clinic dogs than in the other species examined. This is shown in Table 4 which indicates the percentages of *Proteus* isolations in the various species.

Table 4

Species	Percentage <i>Proteus</i> Positive
Canine	45
Avian	11.55
Porcine	11
Bovine	3

EFFECT OF ANTIBIOTICS ON PROTEUS IN VITRO

Penicillin and Streptomycin.

(a) A twenty-four hour broth culture was used. One c.c. culture was added to one c.c. (5,000 units) penicillin.

One c.c. of culture was added to one c.c. (50,000 mg) tomycin

Both mixtures were allowed to stand at room temperature for thirty minutes.

A loopful of each mixture and a loopful of culture alone were streaked on separate sections of a D.C.L.S. plate: There was complete inhibition with streptomycin and partial inhibition with penicillin. Results of incubating for twenty-four hours at 37° C. are shown in Figure 1.

(b) A second test was carried out using a saline slant emulsion in lieu of the broth culture. The amount of streptomycin was reduced to 5,000 mg per c.c. Exposure and incubation periods were the same as in the first test. D.C.L.S. and agar plates were used. This experiment was repeated and the results on each occasion were similar to those obtained in the first test.

Using similar technique dilutions of 500 mg and 50 mg of streptomycin were tested. Complete inhibition did not occur in either of these dilutions.

Testing Antibiosis With Sensitivity Disks.

The comparative inhibitory effect of a number of antibiotics was determined by the use of sensitivity disks (Bacto). The following is a list of the antibiotics tested and the amount in each disk:

Bacitracin	2 units	10 units	20 units
Chloromycetin	10 mcg.	30 mcg.	60 mcg.
Dihydrostreptomycin	0.5 units	1 mcg.	10 mcg.
Penicillin	0.5 units	1 unit	10 units
Streptomycin	0.5 units	1 mcg.	10 mcg.
Terramycin	10 mcg.	30 mcg.	60 mcg.

Agar, blood agar, and D.C.L.S. plates were used in each case. The entire surface was moistened with broth culture of *Proteus*. A number of disks were placed on the surface of each plate. The plates were incubated for twenty-four hours. A comparison was then made of the zones of inhibition. It was found that of the antibiotics tested, Chloromycetin consistently produced the widest zone of inhibition. Streptomycin also produced marked inhibition. Bacitracin produced no visible zone of inhibition. Some of these results are illustrated in figures 2,3, and 4.

PATHOGENICITY AND TREATMENT

The occurrence of this organism in apparently normal individuals has thrown doubt on its importance as a cause of disease, or at least as a primary cause. A number of references have, however, been quoted putting forward evidence that under certain circumstances the organism is associated in some way with disease conditions. For example, we have Craige's contention that the organism is an important cause of disease in dogs. It is evidently an important cause of cystitis in man, but its role in intestinal infections is in some doubt. There are a few reports indicating that disease in young turkeys has been attributed to this organism.

Its isolation here from outbreaks of disease in turkeys and chickens in which there was a considerable mortality and in which usually no other organism was found suggests that it does play an important part in the production of this mortality.

It may be that some predisposing factor is necessary before the organism becomes pathogenic.

It is realized that considerable variations in pathogenicity undoubtedly exist between different strains. In view of the difficulty of fixing the potency of this type of organism, few attempts were made to estimate the number of organisms used in the pathogenicity tests.

A twenty-four hour broth culture of emulsion from an agar slant was usually the inoculum. Cultures grown for ninety-six hours were also used and seemed to be more potent. Isolations from three separate outbreaks have been mixed to form the inoculum used which is called GIM.

Pathogenicity and Treatment in Chickens

The first series of eight experiments were designed to determine in a general way the pathogenicity of the strains GIM and at the same time to note the effect of the administration of streptomycin. The results which are summarized in Table 5 indicate a low pathogenicity. In subsequent experiments a much higher degree of pathogenicity was observed.

Table 5
PATHOGENICITY OF GIM AND EFFECT OF
STREPTOMYCIN

Number	Age	Culture	Route	Treated	Dose	Lived	
						Treated	Untreated
13	3 days	1	c.c. per os	7	1000 units	7	6
10	1 day	1	c.c. per os				10
8	1 day	0.75	c.c. s.c.	4	1000 units	4	0
12	3 days	1	c.c. s.c.	6	1000 units	6	6
16	2 days	0.75	c.c. I.P.	8	1000 units	6	5
12	3 days	0.5	c.c. I.P.	6	5000 units	6	6
2	2 weeks	0.5	c.c. per os				0
2	2 months	0.5	c.c. per os				2

The effect on embryonated chicks was determined by inoculating embryos which had been incubated for seventeen days. Seventeen received 0.1 c.c. of broth culture and seventeen received 0.1 c.c. of sterile broth. Seven of the former and thirteen of the latter hatched. At one week there were four of the former and ten of the latter remaining alive.

A second series of experiments was carried out with a fresh isolation of strains GIM. The results summarized in Table 6 revealed a considerable degree of pathogenicity for young chicks and in one experiment repeated doses of streptomycin proved to be of considerable value in treatment. The method of carrying out these experiments was as follows:

(a) Twenty one-day-old chicks were starved for eighteen hours. A forty-eight hour broth culture of GIM was then mixed with the mash and the drinking water. It was readily taken. After two hours, ten received 1 c.c. streptomycin (50,000 ug) I/M and S/C. All died within two hours. Five of the untreated ten died within three days.

(b) Nineteen two-day old chicks were starved twenty-five hours. A saline emulsion of agar of GIM was given in the food and water. After four hours, nine received 0.5 c.c. streptomycin (500 ug per c.c.). Four of these died within forty-eight hours. The remaining five received a repeat dose of streptomycin. Two of these died. Three of the treated and three of the untreated survived.

(c) Fifteen ten-day-old chicks were starved for twenty-nine hours. Three died during this period. A saline emulsion of agar of GIM was given in the food and water. Six were treated by inoculation into the crop of 5,000 Mg. streptomycin. Only one out of each group died. This again suggests the rapid age resistance that appears to be built up. One or two inoculations of streptomycin do not appear to be satisfactory.

(d) Ten two-day-old chicks were starved for twenty-four hours. They were then inoculated subcutaneously with an emulsion from a ninety-six hour growth on agar (approximately 900,000,000 organisms per c.c.). After one and a half hours, five received 2,500 micro grams of streptomycin subcutaneously. After nineteen hours, four untreated and one treated were dead. The survivors were then fed, and the treated received a second dose of streptomycin. After six hours a further dose of streptomycin was given. The untreated chick died the following day. Three of the treated survived to the sixth day after inoculation when they were all found dead.

(e) Fifty two-day-old chicks were starved for twenty-four hours then inoculated subcutaneously with 0.5 c.c. of a four-day broth culture of GIM. Feeding then recommenced. Streptomycin was repeatedly administered subcutaneously in doses of 2,500 Mg. (0.5 c.c.) at the times indicated in the chart to twenty-five of the chicks.

Hours After Exposure 1½ 6½ 10 22 26 30 35 46 70 78

Treated Group								7	8
Total Mortality	0	1	2	4					
Untreated Group									
Total Mortality	0	5	10	25					

At the twenty-second hour, 100 % of the untreated group had died and 16 % of the treated group. **Proteus** sp. was isolated from two of the chicks which died in the untreated group.

Table 6
PATHOGENICITY AND TREATMENT

No.	Age	Culture Dose	Route	Treated	Dose	Lived	
						Treated	Untreated
20	1 day		Ingestion	10	50,000 Mg.	0	5
19	1 days		Ingestion	9	500 Mg.	3	3
15	13 days		Ingestion	6	500 Mg.	5	6
10	2 days	1 c. c.	S. C.	5	2,500 Mg. repeated	4	0
50	2 days	0.5c.c.	S. C.	25	2,500 Mg. repeated	17	0

A simple *in vitro* experiment was carried out with neomycin which indicated that this antibiotic was markedly inhibitory for *Proteus mirabilis*. The following two experiments were carried out to determine the value of this antibiotic in preventing mortality in chicks artificially infected with our GIM strains. These results are summarized in Table 7.

Fifty three-day-old chicks were starved for eight hours. They were then injected subcutaneously with 0.5 c.c. of a forty-eight hour broth culture of GIM. Two hours later twenty-five chicks each received 0.00125 gm. neomycin subcutaneously. Four hours after the injection the same chicks received 0.000625 gm. Eighteen hours after receiving culture, fifteen of the untreated and one of the treated were dead. Twenty-four hours later a total of nineteen of the controls were dead and only the one of the treated—76 % as against 4 %

Fifty-six three-day-old chicks starved for two hours, then fed usual mash to which had been added about 40 c.c. of a twenty-four hour broth culture GIM. Two hours later twenty-nine each received .00125 gm. neomycin by mouth. After six days, nineteen of the treated and nine of the untreated remained alive, that is, approximately 65.5 % as against 33 %

Table 7
EFFECT OF NEOMYCIN

No.	Age	Culture	Route	Treated	Dose	Lived	
						Treated	Untreated
50	3 days	0.5 c. c.	S. C.	25	.00125 gm.	24	6
					.0006215 gm.		
6	3 days		Ingestion	29	.00125 gm.	19	9

PATHOGENICITY AND TREATMENT IN POULTS

These experiments were of a preliminary nature but the results are included here as a basis for further work.

1. Young poults three to fifteen days old.
Five received 2 c.c. intraperitoneally. All died.
Five received 1 c.c. subcutaneously. Three died. Two received 0.5 c.c. per os. One died. Two received 0.5 c.c. subcutaneously after previously having been starved for twenty-four hours.
Two died.
2. Four poults, five weeks old which had received previous inoculations, were given 1 c.c. intramuscularly. One died.
3. One poult two months old received 1.5 c.c. intravenously. It died.
4. Six four-week old poults received 2.5 c.c. culture intraperitoneally. Three received 10,000 units streptomycin after two hours. All died.
Six received 1 c.c. intraperitoneally. Three of these received 10,000 units streptomycin after two hours and a second dose two hours later. Two of the untreated and one of the treated survived.
5. Twelve two-week-old poults were starved for thirty-six hours and then each given 1 c.c. culture subcutaneously. Two hours later six received 2,500 milligrams streptomycin. After three hours, five of the untreated were dead. The treatment was repeated at this time and $3\frac{1}{2}$ hours later, when three of the treated and the remaining untreated poult were found dead. Two of the three remaining treated poults subsequently died.
6. Five eight-week-old poults received intraperitoneally 1.5 c.c. of an emulsion of GIM containing approximately 2,100,000 organisms per c.c. After two hours, three of the poults received 10,000 units of streptomycin. One of the untreated died.

GROUP AGGLUTINATIONS

Experiments in this field were of a preliminary nature designed to obtain information on which further work might be based. We have seen by reference to several medical and veterinary publications that *Proteus* sp. have an antigenic structure related to a number of organisms of which *Brucella* and *Pullorum* are particularly important in veterinary medicine.

Attempts were made to produce cross agglutinins in guinea-pigs, rabbits, and chickens. The animals received a series of inoculations of *Proteus* strains GIM, a *Pullorum* strain and *Brucella* strain 19.

Sera from guinea-pigs when tested against the several antigens, did not give any indication of cross agglutination (Table 8). Serum dilutions were 1-25, 1-50, 1-100.

Table 8

AGGLUTINATION RESULTS IN GUINEA PIGS

Test Antigen	Strain 19	Immunized with	
		GIM	Pullorum
Br. Abortus	***	---	---
S. Pullorum	---	***	---
<u>Proteus</u> GIM	---	***	---

Serum from hen fifty-four which had received a series of inoculations of *Proteus* GIM, showed slight partial agglutination with a polyvalent pullorum antigen. Serum from hen 19, which had received inoculations of *Brucella* strain 19, showed slight cross agglutination with pullorum antigen. Serum dilutions were 1:50, 1:100 (Table 9).

Table 9

SHOWING SLIGHT CROSS AGGLUTINATION BETWEEN PROTEUS AND PULLORM, AND BRUCELLA AND PULLORUM

Test Antigen	Immunized With		
	GIM	Strain 19	S. Pullorum
Proteus GIM	**	--	--
S. pullorum	II	**	**
Br. abortus	--	--	--

Serum from rabbit 45 and rabbit 7, which received a series of inoculations of *Proteus* GIM, showed no signs of cross agglutination. Serum from rabbit 50, which had received a series of inoculations of *Brucella* strain 19, showed slight cross agglutination with *Proteus* GIM. Serum dilutions were 1.50 and 1.100 (Table 10).

Table 10

SLIGHT CROSS AGGLUTINATION BETWEEN BRUCELLA AND PROTEUS

Test Antigen	Strain 19	Immunized With	
		GIM	GIM
<i>Proteus</i> GIM	*I	**	**
<i>Br. abortus</i>	**	--	--

In one test, serum from Rabbit 50 mentioned above, agglutinated *Brucella* antigen in dilutions of 1 in 1280 and *Proteus* GIM antigen in dilutions of 1 in 320. When this test was repeated, however, only partial agglutination occurred up to 1 in 40 with the latter antigen.

Finally an attempt was made to produce cross agglutination with *Brucella abortus* antigen, by giving two cows a series of inoculations, with *Proteus* GIM. Prior to commencing the inoculations, serum from each cow was tested with *Brucella* antigen, and feces was examined for *Proteus* sp. with negative results. Each cow received a series of seven inoculations of broth culture of *Proteus* at weekly intervals. At the time of the third inoculation and six times subsequently at weekly intervals, blood was drawn and the serum tested for *Brucella* agglutinins at the Brucellosis Laboratory of the Bureau of Animal Industry, Auburn, Alabama. Serum from one cow showed incomplete agglutination in 1 in 40 dilutions at the sixth bleeding. Otherwise the results were negative.

DISCUSSION

The occurrence of members of the genus **Proteus** as pathogenic organisms in various species of animals is apparently controversial. There is some evidence to indicate that in man the genus is responsible for a variety of inflammatory conditions of which cystitis is perhaps the most common. As a cause of enteritis in man, the position of the organism is still in doubt; although a number of cases are on record when its etiological significance seemed to be confirmed.

The isolation of **Proteus** from rectal swabs and other specimens is easily accomplished by the well-founded methods established for the bacteriological examination for enteric organisms. On plate medium no difficulty was encountered in obtaining isolated colonies, Urea broth and urea agar were very useful for separating **Proteus** from other non-lactose fermentors. We were not particularly concerned with the identification of species. Although in a number of instances when this was done with specimens from poult and chicks, the organism was found to be **P. mirabilis**.

The occurrence of the organism in a comparatively high percentage of samples of feces from apparently normal dogs makes an evaluation of its importance as a cause of enteritis in this animal difficult. It has been suggested that the incidence in the stools of normal puppies is low. It may at this time play an important role in the etiology of enteritis. In older animals where there is no other apparent cause of the enteritis, I consider that its isolation is of some significance. The organism may at least be an important contributing factor to the enteritis and should be considered in deciding on the treatment to be adopted. At the Small Animal Clinic in Auburn, Alabama, this information has proved of value in determining a useful method of treatment.

The incidence of the genus in normal chicks is apparently comparatively low. It cannot be considered to be a normal inhabitant of the intestinal tract of young chicks. The organism has, however, not infrequently been isolated in outbreaks of diseases when no other cause has been determined. For example, in one outbreak of disease in chicks in which a heavy mortality occurred, **Proteus** was isolated from each of four chicks killed for postmortem examination. In a number of cases **Proteus** has been the only organism isolated from reactors and suspicious reactors to the **Pullorum** plate test. The possibility of cross agglutination being a factor in some of these cases cannot be ignored.

The rectal samples from cattle and swine were from various age groups and various premises, and the incidence

was comparatively low. It may be deduced that the genus is not commonly a part of the normal intestinal flora of these species. It is worth noting that more than half of the positive samples from swine were in one herd which was garbage fed. It is reasonable to presume that variations in diet do to some extent influence the nature of the bacterial flora of the intestinal tract.

Although not the primary object of this inquiry, the occurrence of **Salmonella** and related genera was noted. It is interesting to observe that in the four animal species from which a total of over four hundred specimens were examined, only one **Salmonella** was isolated and that from the dog. Studies of reports on the incidence of **Salmonella** in dogs in the United States and Great Britain show an incidence varying from 1% to 30%. The highest rate was noted in Florida. The isolation from the dog of a *Paracolony* closely related to the Arizona group is of interest since hitherto this group has not been recorded from the dog.

It was not difficult to cause a high mortality in young chicks and poults by parental inoculation of cultures of **Proteus merabilis**, and also by ingestion, the culture being mixed with the mash. In the latter case, a preliminary period of starvation was enforced to insure that all the subsequent feed would be eaten.

The comparative effect of various antibiotics on **Proteus** is easily demonstrated *in vitro*. This was first studied by adding the antibiotic to a broth culture and subsequently making plate subcultures. Sensitivity disks are also of value in making a rough comparison of inhibitory power on petri plates. By these methods, chlormycetin and neomycin appeared to have the greatest inhibitory power. The effect of two antibiotics was determined *in vivo* in chicks. It was shown with streptomycin and neomycin, particularly the latter, that they were well tolerated by young chicks in dosage sufficient to have a decidedly favourable influence on the condition induced by artificial infection, and to reduce the mortality rate very considerably. In the Small Animal Clinic here, neomycin has proved to be very effective in certain persistent enteric infections of dogs in some of which **Proteus** is involved.

Since **Proteus** has been reported as one of a number of genera which cross agglutinates with **Brucella** serum, a determination of its incidence in certain herds might be of interest. At least one report of the significance of this fact in the serological diagnosis of brucellosis in swine has been noted. In some preliminary experiments in rabbits and hens we were able to demonstrate indications of group agglutinins between these two genera.

CONCLUSIONS

Both in human and veterinary medicine, the role of the genus **Proteus** as a pathogen is found to be controversial. It undoubtedly frequently occurs in some species particularly in the intestinal tract of dogs and perhaps man, without producing symptoms of disease. When no other cause of an enteritis is apparent, however, it may be that this organism has adopted the role of a **Pathogen**. The incidence of **Proteus** in the intestinal tract of normal cattle, and probably swine, is comparatively low. Young chickens can quite easily be artificially infected and killed by **Parenteral** inoculations and by ingestion. It is apparently not a part of the normal intestinal flora of young chicks, and when isolated therefrom in outbreaks of disease, should at least be considered a possible factor in causation.

Neomycin appears to be a most effective antibiotic for use against this organism in chicks.



Figure 1.

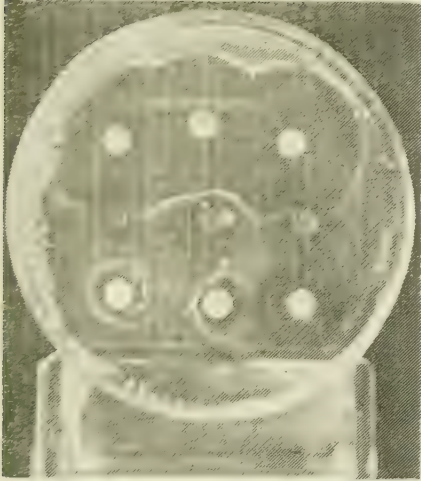


This shows complete inhibition with streptomycin, partial inhibition with penicillin, and the control area.



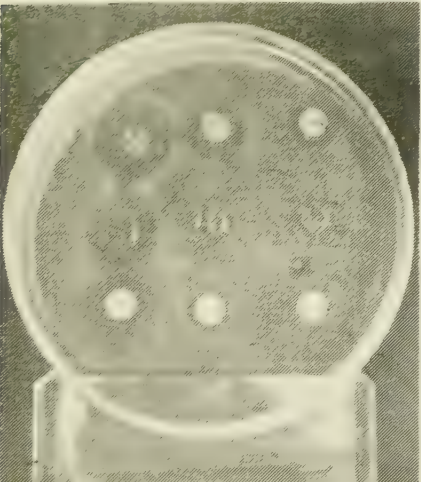
Comparative zones of inhibition with
 Penicillin
 Streptomycin
 Terramycin

Figure 2.



Comparative zones of inhibition with
 Chloromycetin
 Penicillin
 Drhydrostreptomycin

Figure 3.



Comparative zones of inhibition with
 Terramycin
 Penicillin
 Drhydrostreptomycin

Figure 4.

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